Journal of Medical Virology 58:313–320 (1999)

Genotyping of Measles Virus Isolates From Central Europe and Russia

Sabine Santibanez,[1]* Alla Heider,[2] Edith Gerike,[1] Alexander Agafonov,[3] and Eckart Schreier[1]

[1]*Robert Koch Institute, Berlin, Germany*
[2]*Research Institute of Viral Preparations of the Russian Academy of Medical Sciences, Moscow, Russia*
[3]*Research Institute of Molecular Biology of State Scientific Center of Virology and Biotechnology "Vector," Koltsovo, Novosibirsk Region, Russia*

Sequence analysis of 285 nucleotides located on the variable part of the N gene was undertaken on measles virus (MV) samples collected from acutely infected patients in Germany, the Czech Republic, Denmark, Poland, and Russia. Two distinct genotypes (C2 and D6) have circulated in Germany between 1993 and 1996. Isolates of genotype C2 were related to strains reported in Germany before 1993. This genotype was also found in the Czech Republic in 1992 and in Denmark in 1997. The occurrence of genotype D6 in Germany is described below for the first time. In 1998, this genotype was identified in Poland. Genotypes C2 and D6 were also reported in Spain and in the United Kingdom between 1992 and 1996. Therefore, it is concluded that these genotypes are widely distributed over Europe. The analysis of the isolates from Russia revealed that genotype A was present in 1988 in the European part of the country and in 1996 in Siberia. An isolate identified in 1997 in Siberia belonged to genotype D6, which had never been found previously in Russia. We also analysed MV obtained from a case of subacute sclerosing panencephalitis (SSPE) in 1995 in Turkey. A comparison of this sequence with published sequences implied that this SSPE case was associated with a new genetic lineage of MV. ***J. Med. Virol. 58:313–320, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: wild-type strains; N gene; classification; phylogenetic tree; SSPE

INTRODUCTION

Measles, a potentially eradicable disease, still ranks as one of the leading causes of childhood morbidity and mortality. For Europe, the short-term aim proclaimed by the World Health Organisation (WHO) is to reduce measles morbidity and mortality with the objective of eliminating indigenous measles transmission by the year 2007. Surveillance of measles should be strengthened in all countries of this region, with laboratory confirmation playing an increasingly important role as measles incidence declines [European Advisory Group on the Expanded Programme on Immunization, 1997].

The detection of measles virus (MV) circulating in a population is one of the most important functions of surveillance. The qualification of MV circulation requires a method for strain differentiation. Although MV is considered to be serologically monotypic, genetic differences have provided a basis for molecular epidemiological studies. Nucleotide sequence analysis performed world-wide has shown that distinct lineages of wild-type MV exist and co-circulate. Rima et al. [1995] have used the most variable region of the MV genome coding for the C-terminal part of the nucleocapside protein (N) for a phylogenetic analysis of strains circulating world-wide.

Recently, several investigations have described MV circulation in certain countries, such as the United States [Rota et al., 1996], the United Kingdom [Jin et al., 1997], South Africa [Kreis et al., 1997], and Japan [Yamaguchi, 1997]. These investigations have confirmed the findings of Rima et al. [1995] that the different genotypes are not geographically restricted, although some appear to be the predominant "endemic" types in large areas of the world. In Central Europe and Russia, only a small number of isolates have been characterized by sequence analysis. The German strains isolated between 1990 and 1993 belonged to genotype C2 [Rima et al., 1995] and the Russian strain Loss isolated in 1988 in the European part of the country belonged to genotype A [Jin et al., 1996].

In the present study, MV cases were investigated from Germany, the Czech Republic, Denmark, Poland, and Russia. For phylogenetic analysis, a sequence of 285 nucleotides located in the variable region on the N

*Correspondence to: S. Santibanez, National Reference Centre for Measles, Mumps, and Rubella, Robert Koch Institute, Nordufer 20, D-13353 Berlin, Germany. E-mail: SantibanezS@rki.de

Accepted 23 November 1998

TABLE I. MV Strains: Origin of Isolates Characterized in This Work

Isolate, genotype	Location	Isolation year	Sample for sequencing*	Accession number
Prague 1/60, A	Prague, CZH	1960	TC	Y13819
Prague 2/60, A	Prague, CZH	1960	TC	—
Buk 88, A	Moscow, RUS	1988	TC	Y13816
Gag 88, A	Moscow, RUS	1988	TC	—
Il 88, A	Moscow, RUS	1988	TC	—
Nov 1/96, A	Novosibirsk, RUS	1996	TC	Y13818
Nov 2/96, A	Novosibirsk, RUS	1996	TC	Y17030
Nov 3/96, A	Novosibirsk, RUS	1996	TC	Y17031
Prague 231/92, C2	Prague, CZH	1992	NS	—
Prague 243/92, C2	Prague, CZH	1992	NS	Y17027
Prague 244/92, C2	Prague, CZH	1992	NS	Y13820
Prague 256/92, C2	Prague, CZH	1992	NS	—
Prague 329/92, C2	Prague, CZH	1992	NS	Y17028
Hagenow 6/93, C2	Hagenow, DEU	1993	Serum	—
Hagenow 12/93, C2	Hagenow, DEU	1993	Serum	Y13817
Hagenow 15/93, C2	Hagenow, DEU	1993	Serum	—
Jutland 7/97, C2	Jutland, DEN	1997	Urine	Y17024
Funen 9/97, C2	Funen, DEN	1997	Urine	Y17025
Stuttgart/96, C2	Stuttgart, DEU	1996	TS	Y13824
Stuttgart 1/93, D6	Stuttgart, DEU	1993	TC	Y17029
Stuttgart 2/93, D6	Stuttgart, DEU	1993	TC	Y13825
Stuttgart/94, D6	Stuttgart, DEU	1994	TS	Y13823
Berlin/94, D6	Berlin, DEU	1994	TS	Y13814
Berlin 3/96, D6	Berlin, DEU	1996	Serum	—
Berlin 5/96, D6	Berlin, DEU	1996	Serum	—
Berlin 9/96, D6	Berlin, DEU	1996	TS	—
Berlin 22/96, D6	Berlin, DEU	1996	TS	—
Berlin 28/96, D6	Berlin, DEU	1996	TS	Y13815
Berlin 50/96, D6	Berlin, DEU	1996	TS	—
Rostock 59/96, D6	Rostock, DEU	1996	TS	Y13821
Rostock 60/96, D6	Rostock, DEU	1996	TS	—
Rostock 61/96, D6	Rostock, DEU	1996	TS	—
Rostock 62/96, D6	Rostock, DEU	1996	TS	—
Rostock 69/96, D6	Rostock, DEU	1996	TS	—
Nov/97, D6	Novosibirsk, RUS	1997	Saliva	Y17032
Wroclaw 1/98, D6	Wroclaw, POL	1998	Urine	Y17026
Wroclaw 2/98, D6	Wroclaw, POL	1998	Urine	—
SSPE Turk./95, not assign.	Istanbul, TUR	1995	BB	Y13822

*TC, tissue culture; NS, nasal swab; TC, throat swab; BB, brain biopsy.

gene was chosen. The focus of this study was to determine whether the isolates recently identified in Central Europe belong to the same genotypes as those described for Western Europe, and whether the situation in Russia differs from that in Central and Western Europe. Furthermore, the MV sequence obtained from a subacute sclerosing panencephalitis (SSPE) patient from Turkey in 1995 was compared with published MV sequences. Genotyping was performed using the nomenclature recommended for wild-type MV by WHO [1998].

METHODS

Virus Isolates

MV sequences were obtained from clinical specimens and from isolates in tissue culture by RT-PCR. Thirty-seven samples were collected from measles cases confirmed serologically. They included 5 nasal swabs, 12 throat swabs, 4 urine samples, 5 sera, 1 saliva specimen, and 10 supernatants from infected Vero cells. One sample was derived from a brain biopsy of an SSPE patient. Table I lists the origin and the accession number of each of the MV isolates characterized in this work. The origin and the accession numbers of previously described MV strains used for comparison are shown in Table II.

RNA Extraction

RNA was extracted from clinical specimens or from tissue culture using the guanidinium isothiocyanate technique [Chomczinski and Sacchi, 1987].

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

A region located on the N gene was reverse transcribed using Moloney murine leukaemia virus reverse transcriptase and the specific primer MN 1 (1196–1217, 5′ ATTAGGGCAAGAGATGGTAAGG) [Rima et al., 1995; nucleotide positions according to Mori et al., 1993]. A part of the cDNA was amplified by nested PCR using the primer MN 1 and MN 2 (1739–1722, 5′ TATAACAATGATGGAGGG) [Rima et al., 1995] for the first round, and the primer MN 3 (1233–1254, 5′ GT-

TABLE II. MV Strains: Origin of Previously Sequenced Strains Referred to in This Paper

Strain, genotype	Location	Isolation year	Sample for sequencing*	Sequence reference	Accession number
Edm wt, A	USA	1954	TC	Rota et al. (1994a)	U01987
AIK-C (Edmonston-derived vaccine)			TC	Mori et al. 1993	S58435
Chg vaccine	CHN	1957	TC	Rota et al. (1994b)	U03653
Ph26, A	USA	1957	TC	Rota et al. (1994a)	U01991
S191 vaccine	CHN	1960	TC	Rota et al. (1994b)	U03664
Hln, A	FIN	1962	TC	Rota et al. (1994a)	U01996
CAM vaccine	JPN	1968	TC	Rota et al. (1994b)	U03650
Loss, A	RUS	1988	TC	Jin et al. (1996)	—
Jhb2/88, A	SOA	1988	TC	Kreis et al. (1997)	U64583
Jhb2/89, A	SOA	1989	TC	Kreis et al. (1997)	U64584
Jhb38/95, A	SOA	1995	TC	Kreis et al. (1997)	U64585
Mad79, C1	SPA	1979	TC	Rima et al. (1995)	X84868
JM, C2	USA	1977	TC	Taylor et al. (1991)	D01002
WTF, C2	DEU	1990	TC	Rima et al. (1995)	X84872
DL, C2	DEU	1992	TC	Rima et al. (1995)	X84873
Ma92A, C2	SPA	1992	TC	Rima et al. (1995)	X84869
Ma93F, C2	SPA	1993	TC	Rima et al. (1995)	X84871
UK62, C2	UNK	1994	Urine	Jin et al. (1997)	U29315
UK66, C2	UNK	1994	TS	Jin et al. (1997)	U29323
MVO, D1	UNK	1974	TC	Taylor et al. (1991)	D01004
Jhb1/86, D2	SOA	1986	TC	Kreis et al. (1997)	U64580
Jhb1/89, D2	SOA	1989	TC	Kreis et al. (1997)	U64586
Chicago-1, D3	USA	1989	TC	Rota et al. (1994a)	U01977
Canada, D4	CAN	1989	TC	Rota et al. (1994a)	U01976
Rdpt/94, D4	SOA	1994	TC	Kreis et al. (1997)	U64589
CO-94, D5	USA	1994	TC	Rota et al. (1996)	L46728
New Jersey, D6	USA	1994	Clone NJ-1-94-N	Rota et al. (1996)	L46750
Ma94B, D6	SPA	1994	TC	Rima et al. (1995)	X84863
UK74, D6	UNK	1993	TS	Jin et al. (1997)	U29327
UK139, D6	UNK	1992	Urine	Jin et al. (1997)	U29284
UK234, D6	UNK	1995	Blood	Jin et al. (1997)	U29302

*TC, tissue culture; TS, throat swab.

CAGTTCCACATTRGCATCTG) and MN 4 (1648–1631, 5′ GTGTCCGTGTCTGAGCCT) for the second round.

Nucleotide Sequence Analysis

The PCR products were sequenced with the forward and reverse primers for the nested PCR using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit and the 373A DNA-Sequencer (PE Biosystems). Sequence data were analysed using the Sequence Navigator Software (PE Biosystems).

Phylogenetic Analysis

Nucleotide sequences were aligned with the CLUSTAL W program version 1.6 [Thompson et al., 1994] and the construction of the phylogram was made with PHYLIP (Phylogeny Inference Package) Version 3.57c (J. Felsenstein and the University of Washington, 1995).

RESULTS AND DISCUSSION

Most of the sequences presented were obtained from measles patients detected in Germany between 1993 and 1996, including sporadic cases from Berlin (1994) and Stuttgart (1993, 1994), a local outbreak in Hagenow (1993), and a nationwide epidemic in 1996. From the 1996 epidemic, cases came from Berlin, Rostock, and Stuttgart. The sequence information from the Czech Republic included data obtained in the prevaccination era (1960) and from a local outbreak in 1992 in Prague. The two cases from Denmark came from local outbreaks in 1997 in Jutland and Funen, and the two cases from Poland were from a local outbreak in 1998 in Wroclaw. The Russian isolates were obtained from local outbreaks in Moscow (1988) and Siberia (1996, 1997). Furthermore, we analysed the MV sequence obtained from a 12-year-old boy who died from SSPE in Turkey in 1995.

At least two distinct genetic groups of MV have circulated in Central Europe during the nineties. The first group comprises isolates identified in the Czech Republic (Prague 231/92, 243/92, 244/92, 256/92, 329/92), Germany (Hagenow 6/93, 12/93, 15/93, and Stuttgart/96), and Denmark (Jutland 7/97 and Funen 9/97) (Fig. 1). As shown in the phylogram (Fig. 2), these isolates are closely related to the strain WTF.DEU/90, which represents the genotype C2 as a reference strain. The strain DL, as well as the strains Ma92A and Ma93F isolated in Germany in 1992 and in Spain in 1992/93, respectively, also belong to this genotype [Rima et al., 1995]. During the period 1992–1995, genotype C2 was also identified in the United Kingdom (i.e., strains UK62 and UK66) [Jin et al., 1997]. All these observations indicate that in the 1990s, genotype C2 was widespread throughout Europe.

```
Edm wt          ACTGAGGACA AGATCAGTAG AGCGGTTGGA CCCAGACAAG CCCAAGTATC 1360
Prague 1/60     ---------- ---------- ---------- ---------- ----------
Buk 88          ---------- ---------- ---------- ---------- ----------
Nov 1/96        ---------- ---------- ---------- ---------- ----------
Nov 2/96        ---------- ---------- ---------- ---------- ----------
Prague 243/92   --A------- G--------- ---------- ---------T -------G--
Prague 244/92   --A------- G--------- ---------- ---------T -------G--
Prague 329/92   --A------- G--------- ---------- ---------T -------G--
Hagenow 12/93   --A------- G--------- ---------- ---------T -------G--
Stuttgart/96    --A------- G--------- ---------- ---------T -------G--
Jutland 7/97    --A------- G--------- ---------- ---------T -------G--
Funen 9/97      --A------- G--------- ---------- ---------T -------G--
Stuttgart 1/93  ---------- G--------- ------C--- ---------- -------G--
Stuttgart 2/93  ---------- G--------- ------C--- ---------- -------G--
Stuttgart/94    ---------- G--------- ------C--- ---------- -------G--
Berlin/94       ---------- G--------- ------C--- ---------- -------G--
Berlin 28/96    ---------- G--------- ------C--- ---------- -------G--
Rostock 59/96   ---------- G--------- ------C--- ---------- -------G--
Nov/97          ---------- G--------- ------C--- ---------- -------G--
Wroclaw 1/98    ---------- G-----A--- ------C--- ---------- -------G--
SSPE Turk./95   --C------- G--------- ------C--- ---------- ----------

Edm wt          ATTTCTACAC GGTGATCAAA GTGAGAATGA GCTACCGAGA TTGGGGGGCA 1410
Prague 1/60     ---------- ---------- ---------- ---------- ----------
Buk 88          ---------- ---------- ---------- ---------- ----------
Nov 1/96        ---------- ---------- ---------- ---------- ----------
Nov 2/96        ---------- ---------- ---------- ---------- ----------
Prague 243/92   ---------- ---------- A---A-G--- ---------- -G--------
Prague 244/92   ---------- ---------- A---A-G--- ---------- -G--------
Prague 329/92   ---------- ---------- A---A-G--- ---------- -G--------
Hagenow 12/93   ---------- ---------- A---A-G--- ---------- -G------T-
Stuttgart/96    ---------- ---------- A---A-G--- ---------- -G------T-
Jutland 7/97    ---------- ---------- A---A-G--- ---------- -G------T-
Funen 9/97      ---------- ---------- A---A-G--- ---------- -G------T-
Stuttgart 1/93  ---------- ---------- ---------- ------AG-- ----------
Stuttgart 2/93  ---------- ---------- ---------- ------AG-- ----------
Stuttgart/94    ---------- ---------- ---------- ------AG-- ----------
Berlin/94       ---------- ---------- ---------- ------AG-- ----------
Berlin 28/96    ---------- ---------- ---------- ------AG-- ----------
Rostock 59/96   ---------- ---------- ---------- ------AG-- ----------
Nov/97          ---------- ---------- ---------- -T----AG-- ----------
Wroclaw 1/98    ---------- ---------- ---------- ------AG-- --A-------
SSPE Turk./95   ---------- ---------- ---------- ------AG-- ----------

Edm wt          AGGAAGATAG GAGGGTCAAA CAGAGTCGAG GAGAAGCCAG GGAGAGCTAC 1460
Prague 1/60     ---------- ---------- ---------- ---------- ----------
Buk 88          ---------- ---------- ---------- ---------- ----------
Nov 1/96        ---------- ---------- ---------- ---------- ----------
Nov 2/96        ---------- ---------- ---------- ---------- ----------
Prague 243/92   ---------T ---------- --------G- ---------- A---------
Prague 244/92   ---------T ---------- --------G- ---------- A---------
Prague 329/92   ---------T ---------- --------G- ---------- A---------
Hagenow 12/93   ---------T ---------- --------G- ---------- A---------
Stuttgart/96    ---------T ---------- --------G- ---------- A---------
Jutland 7/97    ---------T ---------- --------G- ------A--- A---------
Funen 9/97      ---------T ---------- --------G- ---------- A---------
Stuttgart 1/93  ---------- ---A-C---- -----C---- ---------- ----------
Stuttgart 2/93  ---------- ---A-C---- -----C---- ---------- ----------
Stuttgart/94    ---------- ---A-C---- -----C---- ---------- ----------
Berlin/94       ---------- ---A-C---- -----C---- ---------- ----------
Berlin 28/96    ---------- ---A-C---- -----C---- ---------- ----------
Rostock 59/96   ---------- ---A-C---- -----C---- ---------- ----------
Nov/97          ---------- ---A-C---- -----C---- ---------- ----------
Wroclaw 1/98    ---------- ---A-C---- -----C---- ---------- ----------
SSPE Turk./95   ---------- ---------- ---------G- ------T-- ----------
```

Fig. 1. Alignment based on the nucleotide sequence nt. 1311–1595 of the MV genome. The sequences of the isolates newly described in this work are given. The following isolates show sequence identity: Prague 1/60, 2/60; Buk 88, Gag 88, Il 88; Nov 1/96, 3/96; Prague 244/92, 256/92; Prague 329/92, 231/92; Hagenow 12/93, 6/93, 15/93; Berlin/94, Berlin 28/96, 3/96, 5/96, 9/96, 22/96, 50/96, Rostock 59/96, 60/96, 61/96, 62/96, 69/96; Wroclaw 1/98, 2/98.

```
Edm wt           AGAGAAACCG GGCCCAGCAG AGCAAGTGAT GCGAGAGCTG CCCATCTTCC 1510
Prague 1/60      ---------- ---------- ---------- ---------- ----------
Buk 88           ---------- ---------- ---------- ---------- ----------
Nov 1/96         ---------- ---------- ---------- ---------- ----------
Nov 2/96         ---------- ---------- ---------- ---------- ----------
Prague 243/92    ------C--- ---------- ---------C ---------- ------C---
Prague 244/92    ---------- ---------- ---------C ---------- ------C---
Prague 329/92    ---------- ---------- ---------C ------A--- ------C---
Hagenow 12/93    ---------- ---------- ---------C ---------- ------C---
Stuttgart/96     ---------- ---------- ---------C ---------- ------C---
Jutland 7/97     ---------- ---------- ---------C ---------- ------A---
Funen 9/97       ---------- ---------- ---------C ---------- ------A---
Stuttgart 1/93   --------T- --T------A ---------- --A------- ----------
Stuttgart 2/93   --------T- --T------A ---------- --A------- ----------
Stuttgart/94     --------T- --T------- ---------- --A------- ----------
Berlin/94        --------T- --T------- ---------- --A------- ----------
Berlin 28/96     --------T- --T------- ---------- --A------- ----------
Rostock 59/96    --------T- --T------- ---------- --A------- ----------
Nov/97           --------T- --T------- ---------- --A------- ----------
Wroclaw 1/98     --------T- --T------- ---------- --A------- ----------
SSPE Turk./95    ---------- --T------- ---------- ------A--- T---------

Edm wt           AACCGGCACA CCCCTAGACA TTGACACTGC ATCGGAGTCC AGCCAAGATC 1560
Prague 1/60      ---------- ---------- ---------- ----A----- ----------
Buk 88           ---------- ---------- ---------- ---------- ----------
Nov 1/96         ---------- ---------- ---------- -A-------- ----------
Nov 2/96         ---------- ---------- ---------- ---------- ----------
Prague 243/92    -----A---- ---T------ ---------- ---------T ----------
Prague 244/92    -----A---- ---T------ ---------- ---------T ----------
Prague 329/92    -----A---- ---T------ ---------- ---------T ----------
Hagenow 12/93    -----A---- ---T------ ---------- ---------T ----C-----
Stuttgart/96     -----A---- --TT------ ---------- ---------T ----------
Jutland 7/97     -----A---- --TT------ ---------- ---------T ----------
Funen 9/97       -----A---- --TT------ ---------- ---------T ----------
Stuttgart 1/93   ----A----- ---------- ---------- -------A-A ----------
Stuttgart 2/93   ----A----- ---------- ---------- -------A-A ----------
Stuttgart/94     ----A----- ---------- ---------- -------A-A ----------
Berlin/94        ----A----- ---------- ---------- -------A-A ----------
Berlin 28/96     ----A----- ---------- ---------- -------A-A ----------
Rostock 59/96    ----A----- ---------- ---------- -------A-A ----------
Wroclaw 1/98     ----A----- ---------- ---------- -------A-A ----------
Nov/97           ----A----- ---------- ---------- -------A-A ----------
SSPE Turk./95    ----A----- ---------- ---------- ---------- ----------

Edm wt           CGCAGGACAG TCGAAGGTCA GCTGACGCCC TGCTT 1595
Prague 1/60      ---------- ---------- ---------- -----
Buk 88           ---------- ---------- ---------- -----
Nov 1/96         ---------- ---------- ---------- -----
Nov 2/96         ---------- ---------- ---------- -----
Prague 243/92    ---------- ---------- --------T- ----C
Prague 244/92    ---------- ---------- --------T- ----C
Prague 329/92    ---------- ---------- --------T- ----C
Hagenow 12/93    ---------- ---------- --------T- ----C
Stuttgart/96     ---------- ---------- --------T- ----C
Jutland 7/97     ---------- ---------- --------T- ----C
Funen 9/97       ---------- ---------- --------T- ----C
Stuttgart 1/93   TA--A----- ---------- ---------- ----C
Stuttgart 2/93   T---A----- ---------- ---------- ----C
Stuttgart/94     T---A----- ---------- ---------- --T-C
Berlin/94        T---A----- ---------- ---------- ----C
Berlin 28/96     T---A----- ---------- ---------- ----C
Rostock 59/96    T---A----- ---------- ---------- ----C
Nov/97           T---A----- ---------- ---------- ----C
Wroclaw 1/98     T---A----- ---------- ---------- ----C
SSPE Turk./95    ---------- ---------- ---------- ----C
```

Fig. 1. Continued.

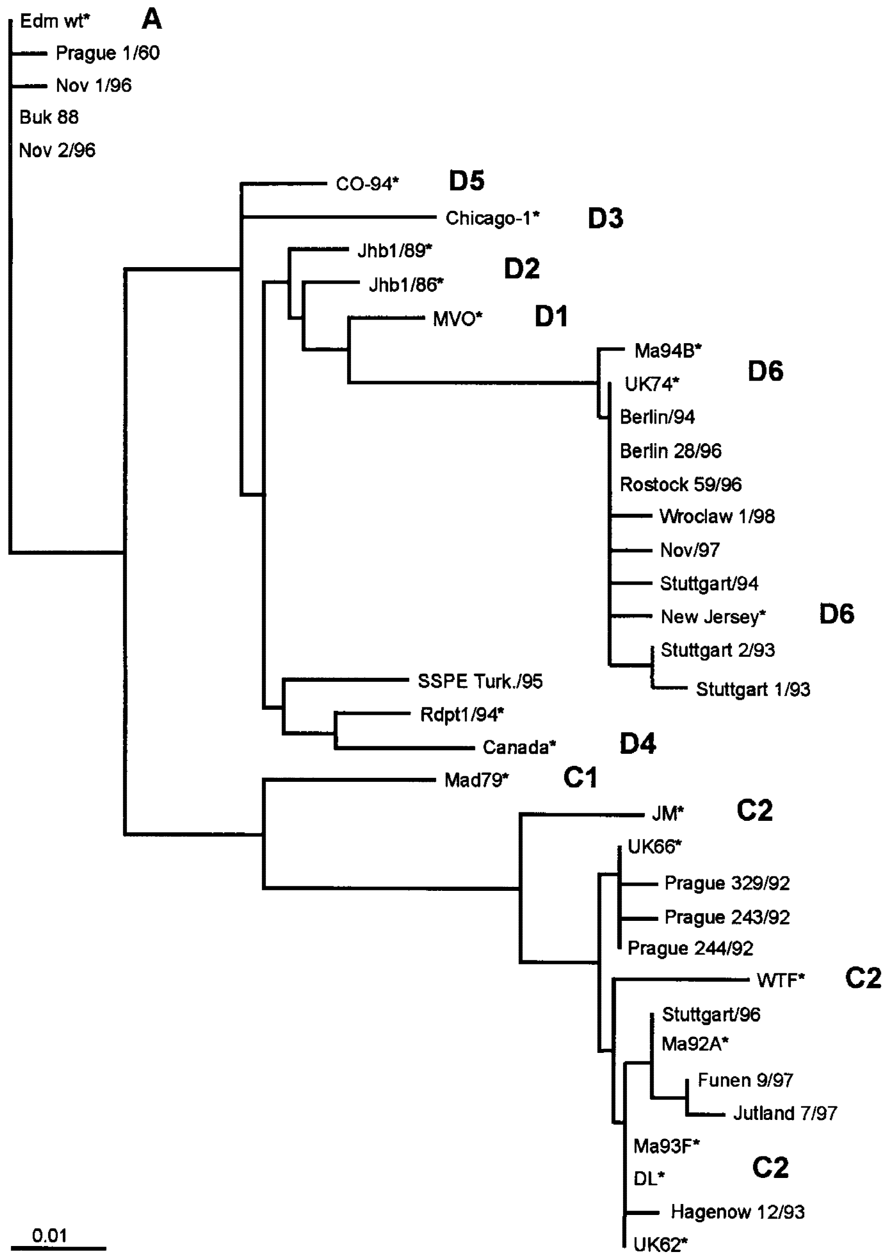

Fig. 2. Unrooted tree diagram (phylogram) based on the nucleotide sequence nt. 1341–1595 of the N gene. The tree was drawn using the programs SEQBOOT and DNADIST in the PHYLIP package. The strains are listed in Tables I and II. Previously described strains are indicated by an asterisk. A, C1, C2, D1, D2, D3, D4, D5, and D6 indicate assignation to genotypes.

The second genetic group of MV in Central Europe comprises isolates collected in Germany (Stuttgart 1/93 and 2/93, Stuttgart/94, Berlin/94, Berlin 3/96, 5/96, 9/96, 22/96, 28/96 and 50/96, and Rostock 59/96, 60/96, 61/96, 62/96, and 69/96) and Poland (Wroclaw 1/98 and 2/98). All these isolates are closely related to the strain New Jersey.USA/94/1 proposed as a reference strain of genotype D6. Recently, genotype D6 was also present in Western Europe. It was detected in the United Kingdom between 1992 and 1995 (i.e., strains UK 139, 74, 234) [Jin et al., 1997] and in Spain in 1994 (i.e., strain Ma94B) [Rima et al., 1995].

The fact that mainly MV belonging to genotypes D6 and C2 have been found in Central and Western Europe during the 1990s leads us to believe that these genotypes dominated here. The isolates obtained from the epidemic that occurred in Germany in 1996 belonged exclusively to these genotypes. Apparently, genotype D6 was the predominant type in this epidemic.

Sequence comparison has shown that after a span of several years, MV with identical nucleotide sequences in the variable genomic part appeared in the same as well as in distant regions within Europe. The strain Ma92A and the isolate Stuttgart/96 as well as the isolates Prague 244/92 and Prague 256/92 and the strain UK66 exhibited identical sequences in genotype C2. In

genotype D6, the strains UK139, 74, 234, the isolate Berlin/94, and all those obtained in Berlin and Rostock in 1996, showed sequence identity. These observations demonstrate the great genetic stability of MV.

During the 1950s and 1960s, only MV belonging to genotype A were isolated world-wide. The following strains are assigned to this group: Edmonston wild-type (Edm wt), Philadelphia 26 (Ph26), and Halonen (Hln), and precursors of the vaccine strains Changchun-47 (Chg), Shanghai-191 (Shg), and CAM-70 (CAM). The characterisation of the isolates Prague 1/60 and Prague 2/60 provides the first data suggesting the presence of genotype A in Central Europe during the prevaccination era. This finding supports the assumption of Rota et al. [1994a] that genotype A may have had a world-wide distribution before vaccination started. In Central and Western Europe in the 1980s and 1990s, excepting a local outbreak in 1993 in Coventry/UK [Outlaw and Pringle, 1995], only MV not belonging to genotype A were identified. The isolates Buk 88, Gag 88, and Il 88 [Tikhonova et al., 1992], and the isolates Nov 1/96, 2/96, and 3/96, show that genotype A has circulated in the European as well as in the Asiatic part of Russia during the past 10 years. Phylogenetic analysis using a part of the noncoding M-F genomic region [Heider et al., 1997] also demonstrated that these Russian isolates belonged to genotype A. Observations of other affected groups also indicate that genotype A has been present in Russia and other regions of the world within the past 10 years. The sequence published by Jin et al. [1996] for the Loss strain isolated in Russia in 1988 corresponds to genotype A. There are also indications that this genotype was present in China in 1993 [Jin et al., 1998]. In South Africa, three strains (Jhb2/88, 2/89, and 38/95) were isolated in 1988/89 and 1995 that also corresponded to genotype A [Kreis et al., 1997]. The following questions can be derived from these results: (a) Do the type A isolates of the last years reflect a circulation of the genotype present in some populations for at least 40 years?; or (b) Do they represent a conversion of a vaccine virus population into a population with characteristics of the wild-type virus?

The occurrence of a MV belonging to clade D in Russia is reported for the first time. Isolate Nov/97 identified in Siberia in 1997 belonged to genotype D6. The close genetic relationship between this Siberian isolate and a number of isolates from different regions of Central and Western Europe including isolates of the epidemic in Germany in 1996 leads to the assumption that there was a connection between Europe and Siberia.

The MV isolate SSPE Turk./95 implies the existence of a previously unobserved phylogenetic lineage of MV. This Turkish isolate has the smallest genetic distance to the strain Rdpt1/94 identified in 1994 in South Africa by Kreis et al. [1997], but the degree of coincidence found between both is only 97.5 % over nt. 1281–1595 of the N gene. Sequence comparisons including other parts of the MV genome will contribute to the determination of the phylogenetic relationship between the isolate SSPE Turk./95 and the existing genetic groups. It is not known when the SSPE patient born in 1983 became infected, so it can only be suspected that the isolate from this patient is derived from an MV that appeared in Turkey after 1983.

The results presented above, coupled with those from the studies of Rima et al. [1995] and Jin et al. [1997], show that Central and Western Europe is a qualitatively uniform region concerning the circulation of genetic groups of MV. As qualitative differences seem to exist between Central and Western Europe on one hand and Russia on the other, an investigation of the European and the Asiatic part of Russia is necessary.

ACKNOWLEDGMENTS

We thank Prof. Dr. G. Enders, Medical Laboratory and Institute of Virology, Infectious Diseases and Epidemiology, Stuttgart, Prof. Dr. C. Huelsse, Landeshygieneinstitut Mecklenburg-Vorpommern, Rostock, Dr. T. Richter, pediatrician, Berlin, Dr. T. Mamaeva, G.N. Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology, Moscow, Dr. L. Dahl, National University Hospital, Copenhagen, Dr. L. Szenborn, Hospital for Infectious Diseases in Childhood of the Medical Academy, Wroclaw, Dr. M. Mrazova, National Institute of Public Health, Prague, and Dr. M. Emeis, Universitätsklinikum Rudolf Virchow, Berlin, who kindly provided clinical specimens.

REFERENCES

Chomczynski P, Sacchi N. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

European Advisory Group on the Expanded Programme on Immunization. 1997. Report on the 13th meeting. WHO Regional Office for Europe, Copenhagen, Denmark.

Heider A, Santibanez S, Tischer A, Gerike E, Tikhonova N, Ignatyev G, Mrazova M, Enders G, Schreier E. 1997. Comparative investigation of the long non-coding M-F genome region of wild-type and vaccine measles viruses. Arch Virol 142:2521–2528.

Jin L, Brown DWG, Ramsay MEB, Rota PA, Bellini WJ. 1997. The diversity of measles virus in the United Kingdom, 1992–1995. J Gen Virol 78:1287–1294.

Jin L, Richards A, Brown DWG. 1996. Development of a dual target-PCR for detection and characterization of measles virus in clinical specimens. Mol Cell Probes 10:191–200.

Jin L, Sun YJ, Ge L, Brown DW. 1998. Characterization of a new genotype of measles virus detected in China and England. Epidemiol Infect 121:691–697.

Kreis S, Vardas E, Whistler T. 1997. Sequence analysis of the nucleocapside gene of measles virus isolates from South Africa identifies a new genotype. J Gen Virol 78:1581–1587.

Mori T, Sasaki K, Hashimoto H, Makino S. 1993. Molecular cloning and complete nucleotide sequence of genomic RNA of the AIK-C strain of attenuated measles virus. Virus Genes 7:67–81.

Outlaw MC, Pringle CR. 1995. Sequence variation within an outbreak of measles virus in the Coventry area during spring/summer 1993. Virus Res 39:3–11.

Rima BK, Earle JAP, Yeo RP, Herlihy L, Baczko K, ter Meulen V, Carabana J, Caballero M, Celma ML, Fernandez-Munoz R. 1995. Temporal and geographical distribution of measles virus genotypes. J Gen Virol 76:1173–1180.

Rota JS, Heath JL, Rota PA, King GE, Celma ML, Brown D, Jin L, Bellini WJ. 1996. Molecular epidemiology of measles virus: identification of pathways of transmission and the implications for measles elimination. J Infect Dis 173:32–37.

Rota JS, Wang ZD, Rota PA, Bellini WJ. 1994b. Comparison of sequences of the H, F, and N coding genes of measles virus vaccine strains. Virus Res 31:317–330.

Rota PA, Bloom AE, Vanchiere JA, Bellini WJ. 1994a. Evolution of the

nucleoprotein and matrix genes of wild-type strains of measles virus isolated from recent epidemics. Virology 198:724–730.

Taylor MJ, Godfrey E, Baczko K, ter Meulen V, Wild TF, Rima BK. 1991. Identification of several different lineages of measles virus. J Gen Virol 72:83–88.

Tikhonova N, Mamaeva T, Naumova M, Leschinskaja E, Volkov M, Martynenko I. 1992. Wild measles virus strain: isolation and identification. Acta Virol 36:557–566.

Thompson JD, Higgins DG, Gibson TJ. 1994. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680.

World Health Organization. 1998. Standardization of the nomenclature for describing the genetic characteristics of wild-type measles viruses. Weekly Epidemiol Record 73:265–272.

Yamaguchi S. 1997. Identification of three lineages of wild measles virus by nucleotide sequence analysis of N, P, M, F, and L genes in Japan. J Med Virol 52:113–120.